REMARKS

Reconsideration and allowance are respectfully requested.

Claims 1-33 and 38 are pending. Non-elected claims 34-37 were withdrawn from consideration by the Examiner. Applicants cancel the non-elected claims without prejudice to future prosecution of that subject matter.

The amendments are fully supported by the original disclosure and, thus, no new matter is added by their entry. Support for "bioluminescence assay" is found on page 5, line 25, of the specification. Amendment of claims 6 and 7 are amended to clarify that the amount of template nucleic acid is determined before or after, respectively, the nucleic acid amplification reaction of ii) (see the paragraph bridging pages 12-13 of the specification). The limitation of claim 16 is incorporated in claim 15.

It was alleged on page 3 of the Action that the Int'l Search Report and Int'l Preliminary Examination Report are not appropriate for printing on the face of an issued patent. Applicants disagree. No such limitation on the types of document that are listed on patents is found in the United States Code, Code of Federal Regulations, or Manual of Patent Examining Procedure. The ISR and IPER are printed documents and acknowledgment that they were considered by printing them on the patent's face is appropriate. Return of an initialed copy of the Form PTO-1449 is again requested. Otherwise, the Examiner is respectfully requested to provide legal authority in support of his allegation.

Additional copies of the documents previously listed for consideration by the Examiner are submitted herewith; new documents are also submitted. They are listed on a Form PTO-1449 (copies of U.S. patent documents are not submitted). The Rule 17(p) fee required for their consideration is attached. For the convenience of the Examiner, the ISR and IPER are also listed on the Form PTO-1449 being submitted herewith. If it is more convenient for her, they can be made of record on the attached. Consideration of the foregoing and return of an initialed Form PTO-1449 to the undersigned is requested pursuant to 37 CFR 1.97(c).

35 U.S.C. 112 – Definiteness

Claims 1-33 were rejected under Section 112, second paragraph, as being allegedly "indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." Applicants traverse.

The term "optional" is deleted from claim 1 because this limitation is not required for patentability. New claim 38 is added to clarify that the optional components are not needed for a bioluminescence assay. Results obtained without using ATP sulphurylase and adenosine 5' phosphosulphate are shown in Figs. 11-12 of the specification.

The term "bioluminescence reaction" is replaced with --bioluminescence assay--, which has antecedent basis. Applicants respectfully disagree with the allegation on page 4 of the Action that it cannot be determined what is encompassed by a bioluminescence assay. The alternatives described in the specification support a broad interpretation for this limitation as different embodiments are encompassed within its scope (see, for example, the optional use of ATP sulphurylase and adenosine 5' phosphosulphate). But this is not evidence of vagueness; instead, it shows the breadth of the term. The skilled artisan understands that "bioluminescence assay for nucleic acid amplification" refers to any assay that measures bioluminescence (i.e., a bioluminescent signal) produced as a result of nucleic acid amplification.

Claims 6-7 and 15 are amended to clarify that they further limit the claims from which they depend. With regard to claims 28-33, the skilled artisan understands that that the components and procedures used in a claimed method, such as the source of the sample and the choice of primers, would be chosen according to the method's use. In accordance thereto, they are amended to require that the method be adapted for the specific use.

Clarification was required on page 5 of the Action for how a decrease in intensity of light output can occur in the claimed method. The Examiner is referred to the light outputs shown in Figs. 2-5, 7 and 10 of the specification, which clearly show that the intensity of light output decreases at certain time points. This is discussed further at pages 32-33 of the specification and is discussed below. Thus, the assertion that "the pyrophosphate production would remain constant in an amplification reaction, and

therefore the production of ATP by ATP sulphurylase, and consequently intensity of light output produced by luciferase via ATP would remain constant as well once reaching maximum" is incorrect as shown by Applicants' working examples.

Claim 15 is amended to provide antecedent basis for "the presence of template nucleic acid in the sample" in claims 17-19.

The specification at page 22, first paragraph, describes nucleic acid amplification reactions which are carried out isothermally. They are those nucleic acid amplification reactions which do not rely on thermocycling for the amplification reaction to proceed. Nucleic acid amplification does not require thermocycling. Claim 26 relates to an isothermal method in which the nucleic acid amplification reaction is carried out at more than one temperature. Claim 27 then further limited the isothermal method by starting at a higher temperature and subsequently dropping to a lower temperature. The subject matter of claims 26-27 is consistent with the definition of "isothermal" as provided in the specification (and known to the skilled artisan) because neither claim requires the nucleic acid amplification reaction to undergo thermocycling.

Applicants request withdrawal of the Section 112, second paragraph, rejections because the pending claims are clear and definite.

35 U.S.C. 102 – Novelty

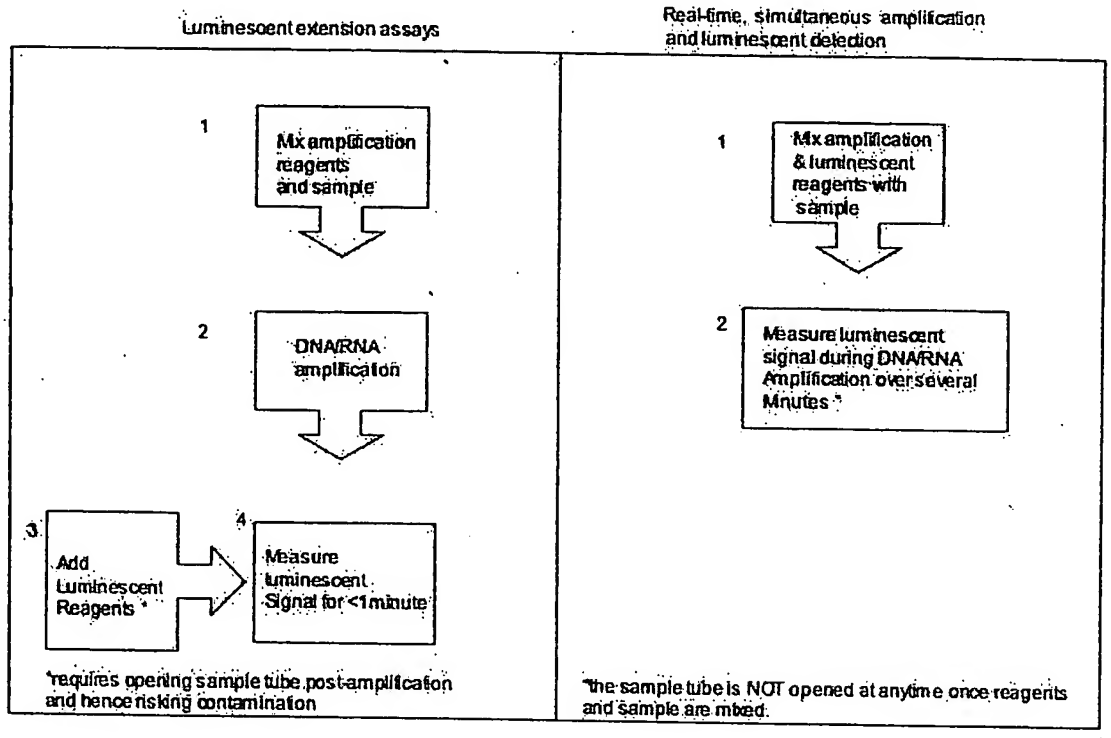
A claim is anticipated only if each and every limitation as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Verdegaal Bros. v. Union Oil Co. of Calif.*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). The identical invention must be shown in as complete detail as is claimed. See *Richardson v. Suzuki Motor Co.*, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989).

Claims 1-19 and 22-33 were rejected under Section 102(b) as allegedly anticipated by Nygren et al. (Anal. Biochem. 288:28-38, 2001). Applicants traverse.

Nygren et al. disclose methods for assessing the amount of a target nucleic acid in a sample. The methods involve co-amplifying the target nucleic acid with at least one competitor nucleic acid molecule that contains a unique discriminatory sequence. Then, following amplification, the relative amounts of the respective amplicons are determined

not require there to be large amounts of template nucleic acid present when the components are added.

The differences between the method of Nygren et al. and a method of Applicants' invention are further illustrated below. Thus, it is clear that the claimed invention is not anticipated by the cited prior art documents.



Primer extension vs. amplification

Further, the Nygren et al. document relates to a method in which a bioluminescence assay is used to monitor the pyrophosphate released by a primer extension reaction which takes place subsequent to the amplification reaction. In contrast, the method of the claimed invention involves detecting the pyrophosphate produced from a nucleic acid amplification reaction. As discussed below, the difference between an amplification reaction used in the claimed invention and the primer extension reaction which is used in the prior art imparts novelty to the subject matter of the pending claims.

Primer extension reactions differ from amplification reactions. In a primer extension reaction, only one round of nucleic acid replication occurs. On the other hand,

amplification of a template nucleic acid is well understood in the art as the repeated copying of a piece of nucleic acid to make multiple copies. For example, *Chambers Science and Technology Dictionary* defines amplification as "The process by which multiple copies of genes of DNA sequences are formed" (emphasis added). Similarly, the Medical Dictionary of MedicineNet.com (last editorial review: 28 Feb 01) defines amplification as "Making multiple copies of a gene or of any sequence of DNA. Repeated copying of a piece of DNA" (emphasis added).

Specifically, the nucleic acid amplification reaction referred to in step ii) of claim 1 of the claimed invention is an *in vitro* technique to produce many copies of a specific nucleic acid sequence, wherein generated copies are themselves further copied leading to an amplification in the copy number of the specific nucleic acid sequence being copied. This is emphasized in the specification at page 6, lines 15-17, which states that:

> The nucleic acid amplification reaction of step ii) can be equated with a "processive" nucleic acid polymerase reaction in that more than one nucleotide addition cycle is carried out without further additions to or manipulation of the buffer components (emphasis added).

Thus, the nucleic acid amplification reaction of step ii) is one in which "more than one" nucleotide addition cycle is carried out and thus differs from the method disclosed in Nygren et al. in which only one nucleotide addition cycle is carried out. Whereas the bioluminescence reaction of the prior art is used to monitor the primer extension reaction, the claimed invention requires that the bioluminescence assay is used to monitor the intensity of light output from the nucleic acid amplification reaction. Consequently, the subject matter of the claims is not anticipated by Nygren et al.

All in one pot

Applicants' invention relates to a method for determining the amount of template nucleic acid in a sample which involves (in part) bringing into association with the sample all the components necessary for nucleic acid amplification and all the components necessary for a bioluminescence assay, wherein these components include at least two primers, and subsequently performing a nucleic acid amplification reaction.

As discussed above, the primer extension reaction of the prior art differs from an amplification reaction and described in Applicants' specification. An amplification reaction is performed in the prior art, but this precedes the primer extension assay, which is an end-point assay used solely to determine whether any or how much amplicon has been produced as a result of the amplification reaction. Consequently, all the components necessary for nucleic acid amplification are not brought into association with the components necessary for the bioluminescence assay in the Nygren et al.

The primer extension reactions described in Nygren et al. are designed to allow quantification by comparison of the signal produced by the target, compared to the signal produced by the competitor. The reactions involve either i) having multiple primers present which are specific, respectively, for the target and competitor nucleic acid(s) and then using a blocking technique, wherein only one nucleotide is added at a time to see whether chain elongation occurs (a method tantamount to pyrosequencing); or ii) having all the nucleotides present, but having only one primer in the reaction mixture that discriminates between the target and competitor nucleic acids, wherein whether chain elongation occurs depends upon whether the primer is a match or a mismatch primer.

Thus the components of the primer extension reactions of Nygren et al. could not in fact be used for an amplification reaction even if the conditions under which the primer extension reaction was performed were altered. This is because all the nucleotides are not present in reaction i) and only one primer is present in reaction ii).

In contrast, an amplification reaction of the present invention requires the presence of "the substrates for the nucleic acid polymerase" and "at least two primers" as is specified by the methods claimed herein.

Consequently, the components used in a method of Applicants' invention differ from the components used in the methods described in the cited prior art. Thus, the subject matter of the claims is not anticipated by Nygren et al.

Thermostable luciferase

The requirement of Applicants' invention for a thermostable luciferase further imparts novelty onto the claimed methods The primer extension assays of Nygren et al. do not use a thermostable luciferase. In contrast, the methods claimed herein require the use of a thermostable luciferase.

The primer extension assays of Nygren et al. were carried out at room temperature and over a short time course (1 min). In contrast, amplification reactions used in a method of the present invention are carried out at elevated temperatures (generally at least 37°C) and take place over a long time course (e.g., 10-15 mins or longer). This requires the use of a thermostable luciferase, as described in Applicants' specification: i.e., a luciferase that is "stable within the temperature range at which the nucleic acid amplification reaction of step ii) is carried out" (see page 7, lines 20-22). The luciferase used in Nygren et al. would not be stable at the elevated temperatures at which amplification reactions take place; neither would it be stable over the longer times required for such reactions. Thus, the luciferase described in Nygren et al. would not be suitable for use in a method of Applicants' invention. Consequently, the claimed methods are not anticipated by Nygren et al.

Claims 1-12, 15-20 and 22-33 were rejected under Section 102(b) as allegedly anticipated by Nyrén et al. (Anal. Biochem. 244:367-373, 1997). Applicants traverse.

The distinctions between Applicants' invention and the prior art discussed above in relation to Nygren et al. apply also to Nyrén et al. In summary, Nyrén et al. document relates to a two-step method in which templates are produced in a first reaction by PCR amplification (see page 368, left-hand column) and are then analyzed in a second reaction involving a primer extension assay to detect single-base changes. A bioluminescence assay is conducted during the primer extension assay (i.e., the second reaction which is separate and distinct from the first reaction), not during the nucleic acid amplification assay (see page 368, right-hand column). Thus, as explained above, the components of the bioluminescence assay are not brought into association with the components necessary for nucleic acid amplification prior to performing the nucleic acid amplification reaction as required by claims 1 and 38.

Claims 1-7, 10-22 and 26-33 were rejected under Section 102(b) as allegedly anticipated by Murray et al. (US 2004/0185457 A1). Applicants traverse.

The Murray et al. document relates to an end-point assay for monitoring the production of amplicon: i.e., the components of the bioluminescence assay are added to (a sample of) the reaction mixture following the amplification reaction. Thus, the components of the bioluminescence assay are not brought into association with the components necessary for nucleic acid amplification prior to performing the nucleic acid amplification reaction as required by claims 1 and 38.

Withdrawal of the Section 102 rejections is requested because the cited documents fail to disclose all limitations of the claimed invention.

Conclusion

Having fully responded to all of the pending objections and rejections contained in this Office Action, Applicants submit that the claims are in condition for allowance and earnestly solicit an early Notice to that effect. The Examiner is invited to contact the undersigned if any further information is required.

Respectfully submitted,

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